FORM PTO-1390 (REV 11-98)	U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 117-284
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/284806 (To be Assigned)
INTERNATIONAL APPLICATION NO. PCT/GB97/02907	INTERNATIONAL FILING DATE 21 October 1997	PRIORITY DATE CLAIMED 21 October 1996

TITLE OF INVENTION

CYTOKINE PRODUCTION INHIBITORS

APPLICANT(S) FOR DO/EO/US

WRIGLEY et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1: ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).

   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).

   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information. International Search Report

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
(To Be Assigned)	PCT/GB97/02907	117-284

17. ☒ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):

-- Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00

-- International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00

-- International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2) paid to USPTO $760.00

-- International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00

-- International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00

			ENTER APPROPRIATE BASIC FEE AMOUNT =			$	840.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).						$	130.00
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total Claims	**18**	-20 =	0	X	$18.00	$	0.00
Independent Claims	**3**	-3 =	0	X	$78.00		0.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)				+$260.00		$	0.00
			TOTAL OF ABOVE CALCULATIONS =			$	**970.00**
Reduction by ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).							0.00
			SUBTOTAL =			$	**970.00**
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).					+		0.00
			TOTAL NATIONAL FEE =			$	**970.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property					+	$	0.00
Fee for Petition to Revive Unintentionally Abandoned Application ($1,210 – Small Entity Fee = $605)						$	0.00
			TOTAL FEES ENCLOSED =			$	**970.00**
						Amount to be: refunded	$
						charged	$

a. ☒ A check in the amount of $970.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Arthur R. Crawford
NAME

25,327 REGISTRATION NUMBER

April 21, 1999 Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

WRIGLEY *et al* Atty Ref:
(National Phase of PCT/GB97/02907)

Serial No. (To Be Assigned) Group:

Filed: Examiner:

For: "CYTOKINE PRODUCTION INHIBITORS"

****

Hon. Commissioner of
Patents and Trademarks,
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir,

In order to place the above-identified application in better condition for examination, please amend the above-identified application as follows:

IN THE CLAIMS

Claims 5, 6, 7, 8, 9 and 10: please delete without prejudice.

Claim 14, line 4 and 5, delete, "or a fatty acid of formula (III) as defined in claim 13".
Please add the following new claims 18 to 24:

- - 18. A method of treating a patient in need of a cytokine production inhibitor, which method comprises administering thereto a therapeutically effective amount of a compound as defined in claim 1.

19. A method according to claim 18 wherein the cytokine production inhibitor is an

IL-1 production inhibitor.

20. A method of treating a clinical condition requiring immunosuppression, which method comprises administering to a patient in need thereof a therapeutically effective amount of a compound as defined in claim 1.

21. A method according to claim 20 wherein said clinical condition is an immunoinflammatory condition.

22. A method according to claim 21 wherein said immunoinflammatory condition is selected from the group consisting of rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma.

23. A method according to claim 20 wherein said clinical condition is a central nervous system disorder.

24. A method according to claim 23 wherein said central nervous system disorder is selected from the group consisting of encephalomyelitis and Alzheimer's disease.--

REMARKS

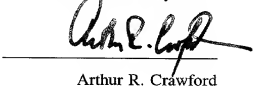
Basis for the new claims is provided in the specification as follows.

Claim	Basis
18	page 11 lines 19-22
19	page 11 line 22
20	page 11 lines 23-24
21	page 11 line 25 and page 12 lines 2-3
22	page 12 lines 2-5

23	page 11 line 25 and page 12 lines 8-9
24	page 12 lines 9-10

Respectfully submitted

Nixon & Vanderhye P.C.



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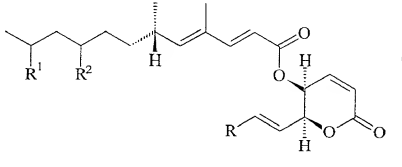
CYTOKINE PRODUCTION INHIBITORS

The present invention relates to 5,6-dihydro-α-pyrones useful as cytokine production inhibitors, to the preparation of these compounds and to pharmaceutical and veterinary compositions containing them.

We have now discovered that fermentation of a strain of the fungus *Phomopsis* sp. in a nutrient medium produces two 5,6-dihydro-α-pyrones esterified in the 5-position with an unsaturated C_{14} fatty acid and also the free C_{14} fatty acid. We have also discovered that fermentation of a strain of the fungus *Paecilomyces* sp. in a nutrient medium produces a useful phomalactone.

The present invention therefore provides a 5,6-dihydro-α-pyrone of formula (I):



(I)

wherein R is CO_2H or CH_3 and each of R^1 and R^2 is H; or R is CO_2H, one of R^1 and R^2 is H and the other is OH; or, when R is CO_2H, a pharmaceutically or veterinarily acceptable salt thereof.

Preferred compounds of the invention are:
3-((5S,6S)-5,6-dihydro-5-((6S)-4,6-dimethyldodeca-2E,4E-dienoyl)-2H-pyran-2-on-6-yl)-prop-2E-enoic acid; and

3-((5S,6S)-5,6-dihydro-5-((6S)-4,6-dimethyldodeca-2E,4E-dienoyl)-2H-pyran-2-on-6-yl)-prop-2E-ene.

The present invention provides a process for the preparation of a 5,6-dihydro-α-pyrone of formula (I) or a pharmaceutically or veterinarily acceptable salt thereof, which process comprises:

(i) fermenting, in a source of carbon, nitrogen and inorganic salts, fungal strain *Phomopsis* sp. 22502 (CBS 313.96) or a mutant thereof which produces a said 5,6-dihydro-α-pyrone;

(ii) isolating a said 5,6-dihydro-α-pyrone from the fermentation broth; and

(iii) if desired, when the isolated 5,6-dihydro-α-pyrone is the compound of formula (I) wherein R is CO_2H, converting the said 5,6-dihydro-α-pyrone into a pharmaceutically or veterinarily acceptable salt thereof.

The compounds of formula (I) have been isolated from a microorganism which we have designated X22502 and which has been identified as a strain of the genus *Phomopsis* (Saccardo) Bubák on the basis of the following morphological data with reference to the description given by SUTTON, B.C., 1980 (The Coelomycetes. Farnham Royal: Commonwealth Agricultural Bureaux):

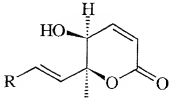
The fungal strain *Phomopsis* sp. (X22502) (CBS 313.96) is a coelomycete isolated from tropical freshwater foam which produces a dense, dark grey-olivaceous (Flora of British Fungi Colour Identification Chart, 1969, Edinburgh: HMSO) mycelium with a white lobate margin at 24°C on 2% malt extract agar with glucose and peptone (MEA: composition per litre of distilled

water: Difco malt extract, 20g; Bacto-peptone, 1g; agar, 20g). After 7 days the mycelium attains a diameter of 2.5 - 3.5cm.

Conidiomatal development is stimulated by exposure to near-UV light. Conidiomata are solitary, carbonaceous, unilocular, ostiolate and measure 1.5 - 2.0mm wide and 1.25 - 2.0mm high. Conidiogenous cells are borne on branched conidiophores which line the conidiogenous cavity. These cells are hyaline, obclavate to cylindrical, integrated, phialidic and measure 16 - 20 μm x 1.5 - 2.0μm. Conidia are hyaline, aseptate and generally of three types: A-conidia (5.5 - 7.5μm x 1.5 - 3.0μm) are ellipsoid to fusiform, usually with acute apices and a guttule at each end; B-conidia (20 - 32μm x <1μm) are hamate and filiform; C-conidia (9.5 - 11.5μm x 1.5 - 3.0μm) are obclavate with acute apices and usually at least three guttules. All three conidial types can be found within a single conidioma. The fluvial origin and observed microscopical characters did not allow further classification to species.

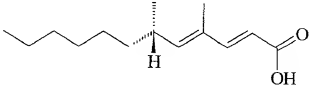
The 5,6-dihydro-α-pyrones of formula (I) are associated primarily with the mycelium on termination of the fermentation. They may be recovered and purified from the medium. The separation and purification of the compounds from the fermentation broth and their recovery can be achieved using solvent extraction followed by chromatographic fractionation. The 5,6-dihydro-α-pyrone of formula (I) in which R is CO_2H may be converted into pharmaceutically or veterinarily acceptable salts by conventional methods. Suitable salts include salts with alkali metals such as sodium or potassium and ammonium salts.

The 5,6-dihydro-α-pyrone of formula (I) wherein R is CH_3 can, alternatively, be produced by the esterification of the phomalactone of formula (II):



(II)

with (6S)-4,6-dimethyldodeca-2E,4E-dienoic acid which is a fatty acid of formula (IIIa):



(IIIa)

Preferably the reaction is carried out in the presence of a dehydrating agent such as DCC (dicyclohexylcarbodiimide) or EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) and dimethylaminopyridine. The reaction is typically carried out in an inert solvent such as dichloromethane or tetrahydrofuran.

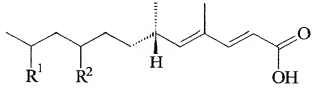
The reagents are generally mixed with stirring for example at a low temperature such as -78°C. The reaction is then allowed to warm to room temperature (20-25°C) and stirred until complete. The reaction may be monitored

chromatographically by thin layer chromatography or reversed phase high performance liquid chromatography and is typically complete within sixteen hours. Other dehydrating agents such as an alkylchloroformate and triethylamine, phenyldichlorophosphate, 2-chloro-1,3,5-trinitrobenzene and pyridine, and chlorosulphonyl isocyanate can also be used under similar conditions.

Alternatively an excess of the phomalactone of formula (II) can be reacted with the acid of formula (IIIa). The water formed can be removed by azeotropic distillation. Suitable solvents include toluene and 1, 4-dioxane. The reaction is typically catalysed by acids such as sulphuric acid and p-toluenesulphonic acid.

It is advantageous to produce the 5,6-dihydro-α-pyrone of formula (I) wherein R is CH_3 by this route as both the phomalactone and the fatty acid can be produced by fermentation in larger quantities than the 5,6-dihydro-α-pyrone of formula (I) wherein R is CH_3.

The fatty acid of formula (IIIa) is one of a group of fatty acids of the following formula (III):



(III)

wherein one of R^1 and R^2 is H and the other is H or OH. These fatty acids can be obtained by fermentation of the fungal strain *Phomopsis sp* 22502 (CBS 313.96) or a mutant thereof.

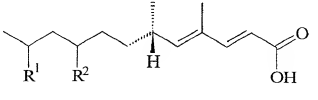
In accordance with the present invention, therefore, the fatty acid of formula (III) can be produced by a process which comprises:

(i) fermenting, in a source of carbon, nitrogen and inorganic salts, strain *Phomopsis* sp. 22502 (CBS 313.96) or a mutant thereof which produces the said fatty acid; and

(ii) isolating the said fatty acid of formula (III) from the fermentation broth.

The fatty acid of formula (III) may, of course, be isolated from the same fermentation broth as the 5,6-dihydro-α-pyrones of formula (I). The fatty acid, like the 5,6-dihydro-α-pyrones, is primarily associated with the mycelium on termination of fermentation.

Some of the fatty acids of formula (III) are novel. The present invention therefore further provides a fatty acid of formula (IIIb):



(IIIb)

wherein one of R^1 and R^2 is H and the other is OH

The phomalactone of formula (II) can be synthesised by methods known in the prior art, for example those disclosed in Krivobok, S. *et al*, Pharmazie, (1994): 49, H8, 605-607; Guirand, P. *et al*, Pharmazie, (1994): 49, H8, 279-281; Krasnoff, S.B. *et al*, J. Chem. Ecol., (1994): 20, 293-302 and Murayama, T. *et al*, Agric. Biol. Chem., (1987): 51, 2055-2060.

The present invention does however provide a new process for the preparation of the phomalactone of formula (II), which process comprises:

(i) fermenting, in a source of carbon, nitrogen and inorganic salts, fungal strain *Paecilomyces* sp. 3527 (CBS 314.96) or a mutant thereof which produces the said phomalactone; and

(ii) isolating the said phomalactone from the fermentation broth.

The phomalactone is found primarily in the culture liquor on termination of the fermentation and may be recovered and purified. The separation and purification of the compound from the fermentation broth and its recovery can be achieved using solvent extraction followed by application of conventional chromatographic fractionations with various chromatographic techniques and solvent systems.

The phomalactone of formula (II) has been isolated from a microorganism which we have designated X3527 and which has been identified as a strain of the genus *Paecilomyces* Bainier on the basis of the following morphological data with reference to the descriptions given by SAMSON, R.A., 1974

(*Paecilomyces* and some allied Hyphomycetes. Studies in Mycology No. 6. Baarn: CBS). The fungal strain *Paecilomyes* sp. (X3527) (CBS 314.96) is an entomogenous hyphomycete isolated from a tropical Lepidoptera pupa which produces white mycelium attaining 4.5 - 5.0cm diameter within 14 days at 25°C on 2% MEA. The aerial mycelium becomes powdery as conidiogenesis occurs and may develop denser concentric zones. A pale-buff-yellow pigmentation frequently develops in aerial and/or submerged mycelium.

Conidiophores are hyaline and smooth-walled with stipe dimensions of 100 - 400μm x 1.5 - 2.5μm and bear single or sparsely clustered phialides. Phialides are produced with the characteristic morphology of *Paecilomyces* Bainier measuring 7 - 20μm long with a lower section inflated to 2 - 2.5μm wide. The phialide neck is often considerably attenuated (<1μm wide) and bent. Phialides of very variable morphology are also produced, e.g. lacking an inflated basal region and/or with an attenuated neck measuring about 20μm long. Conidia are ellipsoid to cylindrical (3.5 - 7μm x 2 - 3μm), smooth-walled, hyaline and borne in conspicuous imbricate dry chains.

Further classification as a *Paecilomyces* anamorph of *Cordyceps* (Fries) Link may be justified on the grounds of the lepidopterous origin of the isolate coupled with the results of numerous studies made by H.C. Evans and R.A. Samson (unpublished data) of entomopathogenic *Paecilomyces* anamorphs of *Cordyceps*.

The strains X22502 and X3527 were deposited by Xenova Group plc of 240 Bath Road, Slough, Berkshire, SL1 4EF, United

Kingdom under the Budapest Treaty at the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, on 19th March 1996 under references X07/64/502 and X08/64/527 respectively. Strain X22502 was assigned the reference number CBS 313.96. Strain X3527 was assigned the reference number CBS 314.96.

The present invention also embraces mutants of the above microorganisms. For example, those which are obtained by natural selection or those produced by mutating agents including ionising radiation such as ultraviolet irradiation, or chemical mutagens such as nitrosoguanidine or the like treatments, are also included within the ambit of this invention.

The invention further provides a biologically pure culture of fungal strain X22502 or X3527 or of a mutant thereof which produces the compounds of the invention. Such cultures are substantially free from other microorganisms. The invention also provides a process for fermenting the fungal strain X22502, X3527 or a said mutant, which process comprises fermenting strain X22502 or X3527 or a said mutant thereof in a source of carbon, nitrogen and inorganic salts.

Assimilable sources of carbon, nitrogen and minerals may be provided by either simple or complex nutrients. Sources of carbon will generally include glucose, maltose, starch, glycerol, molasses, dextrin, lactose, sucrose, fructose, carboxylic acids, amino acids, glycerides, alcohols, alkanes and vegetable oils. Sources of carbon will generally comprise from 0.5 to 10% by weight of the fermentation medium.

Sources of nitrogen will generally include soya bean meal, corn steep liquors, distillers' solubles, yeast

extracts, cottonseed meal, peptones, ground nut meal, malt extract, molasses, casein, amino acid mixtures, ammonia (gas or solution), ammonium salts or nitrates. Urea and other amides may also be used. Sources of nitrogen will generally comprise from 0.1 to 10% by weight of the fermentation medium.

Nutrient mineral salts which may be incorporated into the culture medium include the generally used salts capable of yielding sodium, potassium, ammonium, iron, magnesium, zinc, nickel, cobalt, manganese, vanadium, chromium, calcium, copper, molybdenum, boron, phosphate, sulphate, chloride and carbonate ions.

An antifoam may be present to control excessive foaming and added at intervals as required.

Fermentation can be conducted at temperatures ranging from 20°C to 40°C, preferably 24-30°C. For optimal results, it is most convenient to conduct these fermentations at a temperature in the range 24-26°C. The starting pH of the nutrient medium suitable for producing the compounds can vary from 5.0 to 8.5 with a preferred range of from 5.0 to 7.5.

Small scale fermentations are conveniently carried out by placing suitable quantities of nutrient medium in a flask by known sterile techniques, inoculating the flask with either spores or vegetative cellular growth of the fungal strain, loosely stoppering the flask with cotton wool, and permitting the fermentation to proceed in a constant room temperature of about 25°C on a rotary shaker at from 95 to 300 rpm for 2 to 10 days. The fermentation may also be conducted in static culture on liquid or semi-solid medium.

For larger scale work, it is preferable to conduct the

fermentation in suitable tanks provided with an agitator and a means of aerating the fermentation medium. The nutrient medium is made up in the tank after sterilization and is inoculated with a source of vegetative cellular growth of the fungal strain. The fermentation is allowed to continue for from 1 to 8 days while agitating and/or aerating the nutrient medium at a temperature in the range 20°C to 37°C. The degree of aeration is dependent upon several factors such as the size of the fermenter and agitation speed. Generally the larger scale fermentations are agitated at about 95 to 750 rpm and aerations of about 0.5 to 1.5 VVM (volumes of air per volume of medium per minute).

The separation of the present compounds from the whole fermentation broth and their recovery is carried out by solvent extraction followed by application of chromatographic fractionations with various chromatographic techniques and solvent systems. The present compounds in pure form have thus been isolated in this way.

The 5,6-dihydro-α-pyrones of formula (I) and pharmaceutically and veterinarily acceptable salts of the compound of formula (I) wherein R is CO_2H are inhibitors of the production of cytokines, specifically IL-1β.

These compounds can therefore be used in the treatment of disorders requiring immunosuppression, for example immunoinflammatory conditions and CNS disorders. A human or animal, e.g. a mammal, can therefore be treated by a method comprising administration of a therapeutically effective amount of a compound of formula (I), or a pharmaceutically or veterinarily acceptable salt of the compound of formula (I)

wherein R is CO_2H.

These compounds can be used in the treatment of an immunoinflammatory condition such as rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma. The compounds of the present invention also exhibit pharmacological properties associated with the treatment of other disorders requiring immunosuppression, for example central nervous system (CNS) disorders such as encephalomyelitis and Alzheimer's disease.

The compounds of the present invention can be administered in a variety of dosage forms, for example orally such as in the form of tablets, capsules, sugar- or film-coated tablets, liquid solutions or suspensions or parenterally, for example intramuscularly, intravenously or subcutaneously. The present compounds may therefore be given by injection or infusion.

The dosage depends on a variety of factors including the age, weight and condition of the patient and the route of administration. Typically, however, the dosage adopted for each route of administration for adult humans is 0.001 to 10 mg/kg, most commonly in the range of 0.01 to 5 mg/kg, body weight. Such a dosage may be given, for example, from 1 to 5 times daily orally or by bolus infusion, infusion over several hours and/or repeated administration.

The toxicity of the compounds of the invention is negligible, they can therefore safely be used in therapy.

The compounds of the present invention are formulated for use as a pharmaceutical or veterinary composition also

comprising a pharmaceutically or veterinarily acceptable carrier or diluent. The compositions are typically prepared following conventional methods and are administered in a pharmaceutically or veterinarily suitable form.

For example, the solid oral forms may contain, together with the active compound, diluents, such as lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose, or polyvinyl pyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dye-stuffs; sweeteners; wetting agents such as lecithin, polysorbates, laurylsulphates. Such preparations may be manufactured in known manner, for example by means of mixing, granulating, tabletting, sugar coating, or film coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. In particular a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolise to glucose or which only metabolise a very small amount to glucose. The suspensions and the emulsion may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose or polyvinyl alcohol.

Suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically

acceptable carrier such as sterile water, olive oil, ethyl oleate, glycols such as propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride. Solutions for intravenous injection or infusion may contain a carrier, for example, sterile water which is generally Water for Injection. Preferably, however, they may take the form of a sterile, aqueous, isotonic saline solution. Alternatively, the compounds of the present invention may be encapsulated within liposomes.

The following examples illustrate the invention

Example 1

Batch Fermentation of *Phomopsis* sp. 22502

A 1.5ml cryovial containing 1 ml of macerated vegetative mycelium suspended in a 10% glycerol solution was retrieved from storage at -135°C. A preculture was produced by aseptically placing 1ml of starting material in a 250ml baffled Erlenmeyer flask containing 40ml of nutrient solution S1 and shaken at 240 rpm for 3 days at 25°C.

An intermediate culture was generated by aseptically transferring the preculture to 2L of nutrient solution S1 in a 3L fermenter. The fermenter was agitated at 500rpm, aerated at 0.5vvm, and the temperature controlled at 25°C for 3 days.

A production culture was generated by aseptically transferring an intermediate culture to a 75L fermenter containing 50L of nutrient solution P1. The production fermenter was stirred at 350rpm, aerated at 0.5vvm, and temperature controlled at 25°C. After 5½ days incubation the fermentation was stopped and the culture was harvested.

The nutrient solutions used were as follows, percentages

being by weight:

Nutrient Solution S1: 1.5% glycerol, 1.5% soya bean peptone, 1% D-glucose, 0.5% malt extract, 0.3% NaCl, 0.1% $CaCO_3$, 0.1%: Tween 80, 0.1% Junlon PW110[suppliers: Honeywell and Stein, Sutton, Surrey, U.K.] adjusted to pH 6

Nutrient Solution P1: 3.6% molasses, 0.2% casein hydrolysate, 0.004% phytic acid, 0.09% calcium chloride, 0.1%: Tween 80, adjusted to pH 5

Example 2

Extraction and purification of the 5,6-dihydro-α-pyrones of formula (I) wherein R^1 and R^2 are both H, and the fatty acid of formula (IIIa) from *Phomopsis* sp. 22502

The broth in Example 1 was harvested by filtration using a Schenk Niro 430 filter press, the clarified filtrate was discarded and the retained biomass was extracted with 25L of recirculating methanol for 24 hours. The methanolic extract was harvested *via* filtration through the filter press and evaporated to an aqueous concentrate using a thin film evaporator.

The aqueous concentrate (10L) was then back extracted with 2 x 7L of an ethyl acetate:hexane (1:1) mix. The solvent extracts were pooled and evaporated to a gum under reduced pressure and redissolved in 50ml of ethyl acetate:hexane (1:1). Purification was achieved by normal phase chromatography using a Biotage Flash 75 chromatography system and a Flash 75 KP-Sil silica (32-62 μm 60 Å) column (internal diameter (ID) 7.5 x 30cm length) and an isocratic mobile phase

(ethyl acetate:hexane 1:1 mix, 200ml/min flow rate). 1L fractions were collected and analysed by thin layer chromatography using the same mobile phase as the developing solvent.

The 5,6-dihydro-α-pyrone of formula (I) wherein R is CO_2H (Rf 0.53), the 5,6-dihydro-α-pyrone of formula (I) wherein R is CH_3 (Rf 0.89) and the fatty acid of formula (IIIa) (Rf 0.75) rich fractions were pooled, evaporated to dryness under reduced pressure and subjected to further purification by preparative reversed phase HPLC using a Waters NovaPak C18 (100 Å 5μM) column (ID 2.5 x 20 cm length) and an isocratic mobile phase (80% acetonitrile: 20% water plus 0.1% v/v glacial acetic acid, flow rate 50 ml/min). Wavelength monitoring was at 278nm. The peaks collected at 11-14 minutes, 19-21 minutes and 30-32 minutes were evaporated to dryness to yield the 5,6-dihydro-α-pyrone of formula (I) wherein R is CO_2H (7.5g), the fatty acid of formula (IIIa) (0.35g) and the 5,6-dihydro-α-pyrone of formula (I) wherein R is CH_3 (0.9g), respectively.

Physicochemical data for the three compounds are set out in Tables 1 to 3 below. Tables 2 and 3 show ^1H and ^{13}C NMR assignments respectively.

Table 14

	5,6-dihydro-α-pyrone of formula (I)		fatty Acid of formula (IIIa)
	R is CO_2H	R is CH_3	
DCI-MS *(m/z)*	391 $(MH)^+$	361 $(MH)^+$	225 $(MH)^+$
Molecular formula	$C_{22}H_{30}O_6$	$C_{22}H_{32}O_4$	$C_{14}H_{24}O_2$
UV λ_{max} nm	206, 276	204,274	265
IR (KBr) ucm^{-1}	3391, 2960, 2928, 2855, 1717, 1620, 1570, 1396, 1285, 1250, 1161, 1024, 980.	3390, 2960, 2928, 1720, 1615, 1560, 1250, 1180, 1010, 980.	2900, 2685, 2589 1687, 1618, 1459 1417, 1285, 1207 1028, 984, 940, 852, 700

Table 2

Position	†δH/ppm in MeOH-d4		
	5,6-dihydro-α-pyrone of formula (I)		
	R is CO_2H	R is CH_3	Fatty Acid of formula (IIIa)*
2			
3	6.35 (1H, d, 9.7)	6.29 (1H, d, 9.8)	
4	7.21 (1H, dd, 9.7, 5.6)	7.18 (1H, dd, 9.8, 5.6)	
5	5.64 (1H, dd, 5.6, 3.0)	5.48 (1H, dd, 5.6, 3.1)	
6	5.49 (1H, m)	5.19 (1H, ddq, 7.2, 3.0, 0.9)	
7	6.91 (1H, dd, 15.7, 5.0)	5.72 (1H, ddq, 15.4, 7.2, 1.7)	
8	6.32 (1H, dd, 15.7, 1.8)	6.05 (1H, dqd, 15.4, 6.6, 1.1)	
9		1.84 (3H, ddd, 6.8, 1.7, 0.8)	
1'			
1'-OH			11.70 (1H, br s)
2'	5.88 (1H, d, 15.6)	5.95 (1H, dd, 15.7, 0.6)	5.77 (1H, d, 15.6)
3'	7.41 (1H, dd, 15.6, 0.6)	7.41 (1H, dd, 15.6, 0.8)	7.39 (1H, d, 15.6)
4'			
5'	5.83 (1H, br d, 9.8)	5.83 (1H, br d, 9.2)	5.72 (1H, d, 9.8)
6'	2.66 (1H, m)	2.65 (1H, m)	2.53 (1H, m)
7'-11'	1.3-1.5 (10H, m)	1.4 (10H, m)	1.2-1.4 (10H, m)
12'	0.97 (3H, t, 6.9)	0.95 (3H, t, 6.9)	0.87 (3H, t, 6.7)
13'	1.87 (3H, d, 1.1)	1.81 (3H, d, 1.2)	1.78 (3H, d, 0.8)
14'	1.08 (3H, d, 6.6)	1.09 (3H, d, 6.6)	0.98 (3H, d, 6.6)

* values obtained in $CDCl_3$

† The *J* values are in parenthesis (Hz)

Table 3

Position	δC/ppm in MeOH-d4		
	5,6-dihydro-α-pyrone of formula (I)		
	R is CO_2H	R is CH_3	Fatty Acid of formula (IIIa)
2	164.6	165.5	
3	125.6	126.1	
4	143.4	143.4	
5	64.9	65.6	
6	79.6	81.4	
7	139.6	125.5	
8	128.2	138.8	
9	170.7	18.2	
1'	168.0	168.0	173.1
2'	115.3	115.5	114.7
3'	153.5	153.2	152.1
4'	133.3	133.1	131.2
5'	151.7	151.5	149.8
6'	35.0	34.8	33.2
7'	38.7	38.6	37.1
8'	33.4	33.2	31.8
9'	30.9	30.7	29.3
10'	29.0	28.9	27.3
11'	24.1	23.9	22.5
12'	14.8	14.6	13.9
13'	12.9	12.7	12.1
14'	21.1	20.9	20.2

* values obtained in $CDCl_3$

Example 3

Extraction and purification of the 5,6-dihydro-α-pyrones of formula (I) wherein one of R^1 and R^2 is H and the other is OH, and the fatty acids of formula (IIIb) from *Phomopsis sp* 22502.

The title compounds all possess a hydroxy substituent (as R^1 or R^2) and are therefore more polar analogues of the compounds of formulae (I) and (IIIa) produced as described in Example 2.

The title compounds were isolated from the broth of Example 1 as minor fermentation components, using purification

methods similar to those described in Example 2.

Physicochemical data for the three compounds are set out in the following Tables 4 to 6. Tables 5 and 6 show ^{1}H and ^{13}C NMR assignments, respectively.

Table 4

	Compound of formula (I)		Fatty acid of formula (IIIb)	
	A (R^1=H, R^2=OH)	B (R^1=OH, R^2=H)	C (R^1=H, R^2=OH)	D (R^1=OH, R^2=H)
DCI-MS (m/Z)	424 (MNH_4+) 407 (MH+)	424 (MNH_4+) 407 (MH+)	258 (MNH_4+) 240 (MH+)	258 (MNH_4+) 240 (MH+)
Molecular formula	$C_{22}H_{38}O_7$	$C_{22}H_{38}O_7$	$C_{14}H_{24}O_3$	$C_{14}H_{24}O_3$
UV λ_{max} nm	203, 275	205, 274	267	266
IR (KBr) υ cm^{-1}	3444, 2956, 1723, 1621, 1289, 1249, 1159, 1107, 981	3400, 2900, 1714, 1620, 1286, 1247, 1156, 1106, 980	3255, 2900, 1694, 1627, 1382, 1285 1193, 982	3363, 1692, 1622, 1285, 1198, 1029, 983

Table 5

Position	δH/ppm in MeOH-d4			
	A	B	C	D
2				
3	6.35 (1H,d,9.8)	6.35(1H,d,9.8)		
4	7.21 (1H,dd,9.7, 5.7)	7.21(1H,dd, 9.8, 5.8)		
5	5.67 (1H,dd,5.7, 3.0)	5.67(1H,dd,6.3 ,5.3)		
6	5.52 (1H,m)	5.52 (1H,m)		
7	6.99(1H,dd,15.7, 4.5)	6.98(1H,dd, 15.7, 4.7)		
8	6.30(1H,dd,15.7, 1.8)	6.31 (1H,dd, 15.7, 1.9)		
9				
1'				
2'	5.87 (1H,d,15.5)	5.87 (1H,d, 15.6)	5.87(1H,d, (15.6)	5.85(1H,d, 15.6)
3'	7.40 (1H,d,15.3)	7.40(1H,d, 15.5	7.38(1H,d, 15.9)	7.40(1H,d, 15.7)
4'				
5'	5.84(1H, brd, 9.0)	5.83(1H, brd, 9.9)	5.78(1H,brd, 9.8)	5.75(1H,d, 9.8)
6'	2.67 (1H,m)	2.67 (1H,m)	2.68(1H,m)	2.65(1H,m)
7'	1.3-1.5 (8H,m)	1.3-1.55(8H,m)	1.35-1.6 (8H,m)	1.4-1.6(8H,m)
8'	"	"	"	"
9'	3.50 (1H,m)	"	3.52(1H,m)	"
10'	1.3-1.5 (8H,m)	"	1.35-1.6(8H, m)	"
11'	"	3.77(1H,m)	"	3.78(1H,m)
12'	1.00 (3H,t,7.4)	1.23(3H,d,6.1)	1.00(3H,t, 7.4)	1.20(3H,d, 6.2)
13'	1.87 (3H,s)	1.88(3h,d,1.0)	1.90(3H,d, 0.8)	1.90(3H,s)
14'	1.09 (3H,d,6.6)	1.08(3H,d,6.6)	1.10(3H,d, 6.6)	1.10(3H,d, 6.6)

Table 6

Position	δC/ppm in MeOH-d4			
	A	B	C	D
2	164.0	164.0		
3	125.2	125.2		
4	142.8	142.8		
5	64.2	64.2		
6	78.9	78.9		
7	141.2	141.2		
8	125.4	125.5		
9	168.7	168.8		
1'	167.5	167.6	171.0	171.0
2'	114.8	114.8	116.8	116.8
3'	153.2	153.1	151.6	151.6
4'	132.9	132.9	132.9	132.8
5'	151.2	151.2	149.6	149.7
6'	34.6	34.6	34.5	34.4
7'	31.1	38.3	31.1	38.4
8'	38.4	28.9	38.5	28.7
9'	73.9	27.0	73.9	27.0
10'	38.0	40.2	38.0	40.2
11'	24.8	68.6	24.9	68.6
12'	12.5	23.5	12.6	23.6
13'	10.3	12.5	10.3	12.6
14'	20.7	20.7	20.8	20.9

Example 4

Batch Fermentation of *Paecilomyces* sp. 3527

Starting material of the strain *Paecilomyces* sp. 3527 was generated by suspending a mature slant culture, grown on MEA (2% malt extract, 1.5% agar), in 5ml 10% aqueous glycerol. 1ml of this suspension, in a 1.5ml cryovial, comprises the starting material which was retrieved from storage at -135°C. A preculture was produced by aseptically placing 1ml of starting material in a 250ml baffled Erlenmeyer flask containing 40ml of nutrient solution S2 shaken at 240 rpm for

3 days at 25°C.

An intermediate culture was generated by aseptically transferring the preculture to 2L of nutrient solution S2 in a 3L fermenter. The fermenter was agitated at 500rpm, aerated at 0.5vvm, and the temperature controlled at 25°C for 3 days.

A production culture was generated by aseptically transferring an intermediate culture to a 75L fermenter containing 50L of nutrient solution P2. The production fermenter was stirred at 300rpm, aerated at 0.5vvm, and temperature controlled at 25°C. During the production fermentation the pH was uncontrolled and remained between 5.5 and 6.5. In addition the dissolved oxygen tension remained above 80%. After 5 days incubation the fermentation was stopped and the culture was harvested.

The solutions used were as follows, percentages being by weight:

Nutrient Solution S2: 1.5% glycerol, 1.5% soya bean peptone, 1% D-glucose, 0.5% malt extract, 0.3% NaCl, 0.1% $CaCO_3$, 0.1% Tween 80, 0.1% Junlon PW110 (Honeywell and Stein, Sutton, Surrey, U.K.) adjusted to pH 6

Nutrient Solution P2: 3.65% sucrose, 1.20% glutamic acid (sodium salt), 0.02% K_2HPO_4, 0.98% MES, 0.05% KCl, 0.1% Tween 80, 0.002% $MgSO_4$, 0.002% $CaCl_2$, 2% vitamin mix solution (see below), 0.5% trace elements solution (see below), adjusted to pH 6

vitamin mix solution: 0.0025% thiamine, 0.0025% riboflavin, 0.0025% pantothenate, 0.0025% nicotinic acid, 0.0025% pyridoxine, 0.0025% thioctic acid, 0.00025% folic acid, 0.00025% biotin, 0.00025% cyanocobalamin, 0.00025% *p*-amino

benzoic acid, 0.005% vitamin K, 0.2% Tween 80.

trace elements solution: 0.17% $ZnSO_4$, 0.11% $FeSO_4$, 0.02% $MnSO_4$, 0.006% H_3BO_3, 0.012% $CuSO_4$, 0.005% Na_2MoO_4, 0.005% $CoCl_2$, 0.008% KI.

Example 5

Extraction and purification of the phomalactone from *Paecilomyces* sp. 3527

The fermentation broth from Example 4 was harvested by filtration and the clarified filtrate was divided equally into 3 aliquots and each aliquot was back extracted batch wise with 10L of hexane to remove non-polar impurities. The hexane extracts were separated and discarded. Each batch of filtrate was then back extracted with 2 x 8L of ethyl acetate. The phomalactone and some impurities were extracted into the solvent while many of the more polar impurities remained in the aqueous phase. The ethyl acetate extracts were pooled and concentrated to dryness under reduced pressure and redissolved in 50ml of ethyl acetate.

Purification was achieved by normal phase chromatography using a Biotage Flash 75 chromatography system and a Flash 75 KP-Sil silica (32-62 μm 60Å) column (internal diameter 7.5 x 30cm length) and an isocratic mobile phase (100% ethyl acetate, 200ml/min flow rate). 1L fractions were collected and analysed by thin layer chromatography using ethyl acetate as the developing solvent.

Phomalactone rich fractions (Rf 0.75) were pooled, evaporated to dryness under reduced pressure and subjected to further purification by preparative reversed phase HPLC using

a Beckman 350 HPLC with a Shandon Hyper prep HS BOS C18 (100 Å 12μm) column (internal diameter 10 x 30 cm length) and an isocratic mobile phase (85% water:15% acetonitrile, flow rate 170ml/min). Wavelength monitoring was at 210nm. The peak collected between 6-12 minutes was evaporated to dryness to yield the target phomalactone (6.0g).

Physicochemical data for the phomalactone are set out in Tables 7 to 9 below. Tables 8 and 9 show ^{1}H and ^{13}C NMR assignments respectively.

Table 7

	Phomalactone of formula (II)
DCI-MS *(m/z)*	155 $(MH)^+$
Molecular formula	$C_8H_{10}O_3$
UV λ_{max} nm	200
IR (KBr) υ cm^{-1}	3425, 2923, 2855, 1716, 1629, 1381, 1262, 1157, 1102, 1076, 1034, 969, 830.

Table 8

Position	†δH/ppm in $CDCl_3$
	Phomalactone of formula (II)
2	
3	6.12 (1H, d, 9.6)
4	6.97 (1H, dd, 9.7, 5.2)
5	4.21 (1H, dd, 5.2, 3.2)
6	4.85 (1H, m)
7	6.00 (1H, dqd, 15.3, 6.5, 1.0)
8	5.75 (1H, ddq, 17.0, 6.9, 3.4)
9	1.82 (3H, ddd, 6.1, 1.0)

† The *J* values are in parenthesis (Hz)

Table 9

Position	δC/ppm in $CDCl_3$
	Phomalactone of formula (II)
2	162.9
3	122.8
4	144.3
5	63.1
6	80.9
7	132.9
8	123.8
9	17.8

Example 6

Synthesis of the 5,6-dihydro-α-pyrone of formula (I) wherein R^1 and R^2 are H and R is CH_3 by esterification of the phomalactone with the fatty acid of formula (IIIa).

A solution of the phomalactone (20mg, 0.13mmol 1 eq) in dry dichloromethane (2ml) was added to a stirred solution of the fatty acid of formula (III) (29mg, 0.13mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (24.8mg, 0.13mmol, 1eq) and dimethylaminopyridine (1mg) in dry dichloromethane (5ml) at -78°C. The reaction mixture was allowed to warm to room temperature and was stirred for a further 16 hours. The reaction mixture was washed with saturated ammonium chloride solution (3 X 5ml), water (1 X 5ml) and brine (1 X 5ml). The organic extract was dried over $MgSO_4$, filtered and concentrated *in vacuo* yielding a yellow oil. Purification by preparative reversed phase HPLC yielded the 5,6-dihydro-α-pyrone of formula (I) wherein R^1 and R^2 are H and R is CH_3 (11.1mg). HPLC, TLC, and ^1H NMR analysis showed that this product was identical to the isolated natural product.

Example 7

TNF-α Release from U937 Cells

The effect of the compounds of the invention on TNF-α release was investigated using a known method (Lozanski, G. *et al.*, (1992), J. Rheumatol; 19, 921-26).

The human histolytic lymphoma U937 cell line was obtained from a commercial source (ECACC, Salisbury, UK) and maintained in RPMI 1640 medium supplemented with 2mM L-glutamine and 5% fetal bovine serum. The cells were pretreated with 25 ng/ml PMA for six hours and then exposed to dose ranges of the compound to be tested followed by the addition of 1 ng/ml LPS.

After 18 hours incubation at 37°C with 5% CO_2 the cell culture supernatants were harvested and stored at -70°C, until required for determination of TNF-α secretion by Dissociation Enhanced Lanthanide Fluorescence Immuno Assay (DELFIA). The effect of the compounds on cell cytotoxicity was measured using the tetrazolium salt, XTT (2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide salt) and effects on protein synthesis were determined by investigation of [^{3}H] Leucine uptake.

In this test the compounds of the invention were found to inhibit TNF-α release at concentrations of from 10 to 0.1 μM (Table 10). At this concentration range, the compounds were not toxic and showed no effect on protein synthesis. The IC_{50}(μM) values for LPS-induced TNF production in U937 cells for the 5,6-dihydro-α-pyrones for formula (I) are given in Table 11.

Example 8

IL-1 Release from Human Monocytes

The effect of the compounds of the invention on IL-Iβ release was investigated using a known method (Bakouche, O. *et al.*, (1992), J. Immunol: 148, 84-91). Human monocytes were purified by elutriation from Buffy coats obtained from normal healthy donors after the separation of peripheral blood mononuclear cells (PBMC) on Lymphoprep. The freshly isolated monocytes were suspended in RPMI 1640 supplemented with 5% FBS and exposed to dose ranges of the compound to be tested followed by the addition of 1 ng/ml LPS. Cells were incubated for 18 hours at 37°C with 5% CO_2 and the cell culture supernatants harvested and stored at -70°C. Effects on the production of IL-1β were determined using an ELISA.

The compounds of the invention were found to inhibit the release of IL-1β at concentrations of from 0.2 to 10 μM (Table 12). At this concentration range the compounds were not toxic to monocytes. The IC_{50} (μM) values for LPS-induced IL-1β production for the 5,6-dihydro-α-pyrones of formula (I) are given in Table 11.

Table 10: Inhibition of TNF-α release

	Concentration μM	% Inhibition of TNF-α
R in formula (I) is CO_2H	8	107
	2	100
	0.5	77
	0.12	47
R in formula (I) is CH_3	2.8	99
	0.7	90
	0.17	61
	0.04	20
	0.01	0.85

Table 11: IC_{50}s for inhibition of LPS-induced cytokine production by the 5,6-dihydro-α-pyrones of formula (I)

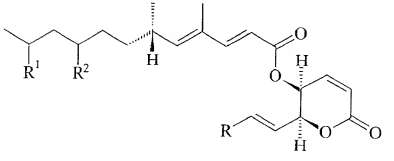
		IC_{50} (μM)	
R^1, R^2 in formula (I)	R in Formula (I)	LPS-induced TNF production in U937 cells	LPS-induced IL-β production (monocytes)
H,H	CO_2H	2	2
H,H	CH_3	0.08	0.19
H, OH	CO_2H	32	-
OH,H	CO_2H	31	-

Table 12: Inhibition of the release of Il-1β

	Concentration μM	% Inhibition of IL-1β
R in formula (I) is CO_2H	20	99
	2	81.5
	0.5	14
R in formula (I) is CH_3	5.5	88
	1.1	72
	0.22	41
	0.044	23

CLAIMS

1. A 5,6-dihydro-α-pyrone of formula (I)



(I)

wherein R is CO_2H or CH_3 and each of R^1 and R^2 is H; or R is CO_2H, one of R^1 and R^2 is H and the other is OH; or, when R is CO_2H, a pharmaceutically or veterinarily acceptable salt thereof.

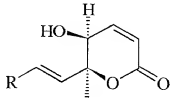
2. A process for the preparation of a 5,6-dihydro-α-pyrone of formula (I) as defined in claim 1 or a pharmaceutically or veterinarily acceptable salt thereof, which process comprises:

(i) fermenting, in a source of carbon, nitrogen and inorganic salts, fungal strain *Phomopsis* sp. 22502 (CBS 313.96) or a mutant thereof which produces a said 5,6-dihydro-α-pyrone;

(ii) isolating a said 5,6-dihydro-α-pyrone from the fermentation broth; and

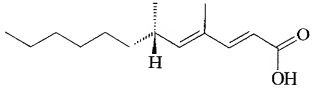
(iii) if desired when the isolated said 5,6-dihydro-α-pyrone is the compound of formula (I) wherein R is CO_2H, converting the said 5,6-dihydro-α-pyrone into a pharmaceutically or veterinarily acceptable salt thereof.

3. A process for the preparation of a 5,6-dihydro-α-pyrone of formula (I), as defined in claim 1, wherein R is CH_3, which process comprises esterifying the phomalactone of formula (II):



(II)

with a fatty acid of formula (IIIa):



(IIIa)

4. A pharmaceutical or veterinary composition comprising a pharmaceutically or veterinarily acceptable carrier or diluent and, as active ingredient, a compound as claimed in claim 1.

5. A compound according to claim 1 for use in a method of treatment of the human or animal body by therapy.

6. A compound according to claim 5 for use as a cytokine production inhibitor.

7. A compound according to claim 6 for use as an IL-1 production inhibitor.

8. A compound according to claim 6 for use in the

treatment of an immunoinflammatory condition.

9. A compound according to claim 8 for use in the treatment of rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease or asthma.

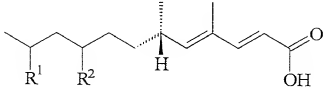
10. A compound according to claim 6 for use in the treatment of a central nervous system disorder.

11. A process for the preparation of the phomalactone of formula (II) defined in claim 3, which process comprises:

(i) fermenting, in a source of carbon, nitrogen and inorganic salts, fungal strain *Paecilomyces* sp. 3527 (CBS 314.96) or a mutant thereof which produces the said phomalactone; and

(ii) isolating the said phomalactone from the fermentation broth.

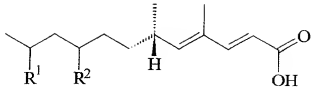
12. A fatty acid of formula (IIIb):



(IIIb)

wherein one of R^1 and R^2 is H and the other is ~~H or~~ OH.

13. A process for the preparation of a fatty acid of formula (III):



(III)

wherein one of R^1 and R^2 is H and the other is H or OH.

which process comprises:

(i) fermenting, in a source of carbon, nitrogen and inorganic salts, fungal strain *Phomopsis* sp. 22502 (CBS 313.96) or a mutant thereof which produces the said fatty acid; and

(ii) isolating the said fatty acid from the fermentation broth.

14. A biologically pure culture of fungal strain *Phomopsis* sp. 22502 (CBS 313.96) or a mutant thereof which produces a 5,6-dihydro-α-pyrone of formula (I) as defined in claim 1 or a fatty acid of formula (III) as defined in claim 13.

15. A biologically pure culture of fungal strain *Paecilomyces* sp. 3527 (CBS 314.96) or a mutant thereof which produces a phomalactone as defined in claim 3.

16. A process for fermenting fungal strain *Phomopsis* sp. 22502 (CBS 313.96) or a mutant thereof as defined in claim 13, which process comprises fermenting strain *Phomopsis* sp. 22502 (CBS 313.96) or a said mutant thereof in a source of carbon, nitrogen and inorganic salts.

17. A process for fermenting fungal strain *Paecilomyces* sp. 3527 (CBS 314.96) or a mutant thereof as defined in claim 14, which process comprises fermenting strain *Paecilomyces* sp.

3527 (CBS 314.96) or a said mutant thereof in a source of carbon, nitrogen and inorganic salts.

DECLARATION FOR NON-PROVISIONAL PATENT APPLICATION*

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. beneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

Cytokine Production Inhibitors

and for which a patent application:
☐ is attached hereto and includes amendment(s) filed on *(if applicable)*
☒ was filed in the United States on April 21, 1999 as Application No.09/284,806 *(for declaration not accompanying application)*
with amendment(s) filed on *(if applicable)*
☐ was filed as PCT international Application No. on and was amended under PCT Article 19 on *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
GB9621859.9	Great Britain	21 Oct 1996	YES ☒ NO ☐
PCT/GB97/02907	PCT	21 Oct 1997	YES ☒ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

PROVISIONAL APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information known to me which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

NON-PROVISIONAL APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

* for use only when the application is assigned to a company, partnership or other organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

201

FULL NAME OF INVENTOR	LAST NAME: Wrigley	FIRST NAME: Stephen	MIDDLE NAME: Keith
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 201: Stephen Wrigley		DATE: 14 May 2001

202

FULL NAME OF INVENTOR	LAST NAME: Bahl	FIRST NAME: Sangeeta	MIDDLE NAME:
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough GBN	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
2-10	SIGNATURE OF INVENTOR 202: Bahl		DATE: 17th May 2001

203

FULL NAME OF INVENTOR	LAST NAME: Guilani	FIRST NAME: Roya	MIDDLE NAME: Mansour Sadeghi
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 203		DATE

204

FULL NAME OF INVENTOR	LAST NAME: Moore	FIRST NAME: Michael	MIDDLE NAME:
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 204		DATE

205

FULL NAME OF INVENTOR	LAST NAME: Katzer	FIRST NAME: Werner	MIDDLE NAME: Albert
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: Germany
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 205		DATE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

201

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Wrigley	Stephen	Keith
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 201: Stephen Wrigley		DATE: 14 May 2001

202

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Bahl	Sangeeta	
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 202		DATE

203

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Guilani	Roya	Mansour Sadeghi
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough GBN	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
3-00	SIGNATURE OF INVENTOR 203: Roya Sadeghi		DATE: 18 May 2001

204

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Moore	Michael	
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 204		DATE

205

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Katzer	Werner	Albert
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: Germany
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 205		DATE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

201

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Wrigley	Stephen	Keith
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks GBN	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 201: Stephen Wrigley		DATE: 14 May 2001

202

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Bahl	Sangeeta	
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 202		DATE

203

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Guilani	Roya	Mansour Sadeghi
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 203		DATE

204

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Moore	Michael	
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough GBN	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 204: MMoore		DATE: 17 May 2001

205

	LAST NAME	FIRST NAME	MIDDLE NAME
FULL NAME OF INVENTOR	Katzer	Werner	Albert
RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: Germany
POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	SIGNATURE OF INVENTOR 205		DATE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

201	FULL NAME OF INVENTOR	LAST NAME: Wrigley	FIRST NAME: Stephen	MIDDLE NAME: Keith	
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks	COUNTRY OF CITIZENSHIP: British	
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom	ZIP CODE: SL1 4EQ
		SIGNATURE OF INVENTOR 201: Stephen Wrigley		DATE: 14 May 2001	
202	FULL NAME OF INVENTOR	LAST NAME: Bahl	FIRST NAME: Sangeeta	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British	
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom	ZIP CODE: SL1 4EQ
		SIGNATURE OF INVENTOR 202		DATE	
203	FULL NAME OF INVENTOR	LAST NAME: Guilani	FIRST NAME: Roya	MIDDLE NAME: Mansour Sadeghi	
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British	
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom	ZIP CODE: SL1 4EQ
		SIGNATURE OF INVENTOR 203		DATE	
204	FULL NAME OF INVENTOR	LAST NAME: Moore	FIRST NAME: Michael	MIDDLE NAME:	
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: British	
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom	ZIP CODE: SL1 4EQ
		SIGNATURE OF INVENTOR 204		DATE	
205	FULL NAME OF INVENTOR	LAST NAME: Katzer	FIRST NAME: Werner	MIDDLE NAME: Albert	
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks, United Kingdom	COUNTRY OF CITIZENSHIP: Germany	
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough GBN	STATE OR COUNTRY: Berkshire, United Kingdom	ZIP CODE: SL1 4EQ
		SIGNATURE OF INVENTOR 205		DATE: 28 May 2001	

206	FULL NAME OF INVENTOR	LAST NAME: Martin	FIRST NAME: Steven	MIDDLE NAME: Michael
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks GBN	COUNTRY OF CITIZENSHIP: British
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	6-00	SIGNATURE OF INVENTOR 206: SMartin		DATE: 14 May 2001
207	FULL NAME OF INVENTOR	LAST NAME: Kau	FIRST NAME: David	MIDDLE NAME: Andrew
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks GBN	COUNTRY OF CITIZENSHIP: British
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire United Kingdom; ZIP CODE: SL1 4EQ
	7-00	SIGNATURE OF INVENTOR 207: D - Kau		DATE: 14 May 2001
208	FULL NAME OF INVENTOR	LAST NAME: Whiting	FIRST NAME: Andrew	MIDDLE NAME: Jonathan
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks	COUNTRY OF CITIZENSHIP: British
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough GBN	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	8-00	SIGNATURE OF INVENTOR 208:		DATE: 14 MAY 2001
209	FULL NAME OF INVENTOR	LAST NAME: Robinson	FIRST NAME: Neil	MIDDLE NAME:
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks	COUNTRY OF CITIZENSHIP: British
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough GBN	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	9-00	SIGNATURE OF INVENTOR 209: Neil Robinson		DATE: 5 June 2001
210	FULL NAME OF INVENTOR	LAST NAME: Hayes	FIRST NAME: Martin	MIDDLE NAME: Alistair
	RESIDENCE & CITIZENSHIP	CITY: Slough	STATE OR FOREIGN COUNTRY: Berks GBR	COUNTRY OF CITIZENSHIP: British
	POST OFFICE ADDRESS	STREET: 545 Ipswich Road	CITY: Slough	STATE OR COUNTRY: Berkshire, United Kingdom; ZIP CODE: SL1 4EQ
	10-00	SIGNATURE OF INVENTOR 210: Martin Hayes		DATE: 21 May 2001.

211					
	FULL NAME OF INVENTOR	LAST NAME Mander	FIRST NAME ~~Thomas~~	MIDDLE NAME Haydn	
	RESIDENCE & CITIZENSHIP	CITY Slough	STATE OR FOREIGN COUNTRY Berks	COUNTRY OF CITIZENSHIP British	
	POST OFFICE ADDRESS	STREET 545 Ipswich Road	CITY Slough	STATE OR COUNTRY Berkshire, United Kingdom	ZIP CODE SL1 4EQ
	11-00	SIGNATURE OF INVENTOR 211		DATE 21.05.01 (21 MAY '01)	

06-06-00

529 Rec'd PCT/PTO 05 JUN 2000
09/284806

Express Mail No. EL 501 632 617 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

09/284806

Application of: Wrigley et al.

Serial No.: 09/284,806

Filed: April 21, 1999

For: Cytokine Production Inhibitors

Group Art Unit: To Be Assigned

Examiner: To Be Assigned

Nixon & Vanderhye P.C.
1100 North Glebe Road - 8th Floor
Arlington, VA 22201-4714

Attorney Docket No.: 117-284

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, DC 20231

S I R:

The undersigned attorney of record in the above-identified application hereby appoints:

Laura A. Coruzzi (Reg. No. 30,742)
T. Christopher Tsang (Reg. No. 40,258)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711

as associate attorneys to prosecute the said application, to make alterations and amendments therein, and to transact all business in the Patent and Trademark Office in connection therewith.

All correspondence relating to this application should be addressed to the associate attorneys whose address is PENNIE & EDMONDS LLP, 1155 Avenue of the Americas, New York, New York 10036.

Respectfully submitted,

Nixon & Vanderhye P.C.
Attorneys for Applicants

Date: 5/31/00
Telephone: (703) 816-4006

Arthur R. Crawford — 25,327 (Reg. No.)

Express Mail No. EL 501 632 617 US

09/284806

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Wrigley et al.

Serial No.: 09/284,806 — Group Art Unit: To Be Assigned

Filed: April 21, 1999 — Examiner: To Be Assigned

For: Cytokine Production Inhibitors

Pennie & Edmonds LLP
1155 Avenue of the Americas
New York, NY 10036

Attorney Docket No.: 9993-018
(formerly 117-284)

NOTIFICATION OF CHANGE OF CORRESPONDENCE ADDRESS

Assistant Commissioner for Patents
Washington, DC 20231

JUL 9 2000

S I R:

An Associate Power of Attorney appointing Laura A. Coruzzi and T. Christopher Tsang as associate attorneys for applicants is filed on even date herewith. Please send all correspondence relating to this application to the Associate Attorney of record, Laura A. Coruzzi, whose address is:

Laura A. Coruzzi (Reg. No. 30,742)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711

Respectfully submitted,

Pennie & Edmonds LLP
Associate Attorneys for Applicants

Date: June 5, 2000
Telephone: (212) 790-6431

Laura A. Coruzzi — 30,742 (Reg. No.)